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Effect of Electric Field Induced Perturbation of the Distribution of Ions Near the Cell Surface on Migration of Charged Membrane Components

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The Effect of Electric Field Induced Perturbation of the Distribution of Ions Near the Cell Surface on Migration of Charged Membrane Components

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It has been demonstrated that an externally applied electric field perturbs the distribution of some of the macromolecules in biological membranes. Various electrostatic, hydrodynamic and structural forces resulting from the external field influence the movement of intramembraneous particles. This study investigates one of those forces, that due to the polarization of the ion distribution near the cell surface.

A model is proposed based on a continuous charge distribution and the electric field due to the polarization of the ion distribution is calculated at any point on the cell surface. Shortly after an external field (E_0) is applied, parallel to the surface on which the cell is fixed, the field due to the ion distribution is $-AE_0 \sin\theta'$, at a point (a, θ', ϕ') where $qaE_0 \ll kT$, a is the radius of the cell and q is the charge of an ion near the surface. From this model we found that for a 0·15 molar monovalent salt solution, A varies from 0·16 to 1·80 as the surface potential of the cell varies between 10 and 80 mV. For larger fields, $qaE_0 \gtrsim kT$, the angular dependence is more complicated but the general conclusions are still valid. Results for other salt solutions and slightly different models are also given. The advantage of using discrete charges and the difficulties of incorporating them into the model are discussed.

It is concluded that the force due to ion distribution polarization will have a significant effect on the movement of charged membrane components and it offers a possible explanation for the movement of charged intramembraneous molecules in a direction not expected when considering only the external field.

1. Introduction

Externally applied electric fields perturb the spatial distribution of some of the charged components within biological membranes (Poo, 1981; Jaffe, 1977; Sowers & Hackenbrock, 1981). This phenomenon has been used to investigate the dynamic aspects of membrane structure and function (Poo, 1981; Jaffe, 1977; Sowers & Hackenbrock, 1981; Poo & Robinson, 1977;

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Zagyansky & Jard, 1979). It can also provide information about the effects of electromagnetic fields on biological systems.

Physical models have been used successfully to interpret the relaxation of the spatial distribution of membrane components after the external field has been removed (Zagyansky & Jard, 1979; Poo *et al.* 1979). However, it has been more difficult to interpret the equilibrium distribution of the observed components during the application of the field. In fact, it is not always possible to predict the direction of the increased accumulation of charged membrane components. It has been suggested that concanavalin A and acetylcholine receptors accumulate at the side of the cell nearest the negative pole because they are positively charged or less negatively charged than other displacable membrane components (Poo *et al.*, 1979). Recently, an alternative explanation for the migration of molecules in the membrane in a direction unexpected from electrostatic considerations has been provided (McLaughlin & Poo, 1981). In their model, the molecules move in response to a hydrodynamic force resulting from electro-osmotic flow of fluid parallel to the cell surface. This force may cause even negatively charged macromolecules to accumulate on the side of the cell near the negative pole if the ζ-potential of a part of the molecule far (>2 nm) from the cell surface is less negative than the ζ-potential of the cell surface. Both of these interpretations are plausible but difficult to quantitate.

In this paper, the effect of the external field on the interaction between charged intramembraneous molecules and positive ions (counter-ions) trapped near the surface by the large electrostatic field of the negatively charged cell is presented as an alternative explanation of these results. It will be shown that the redistribution of these ions can provide a major contribution to the force induced by an external field on charged molecules within cell membranes and may even cause negatively charged receptors to migrate towards the negative pole. Further, consideration of this model provides insight into the mechanisms for biological effects of external electromagnetic fields.

In order to explain the low frequency dielectric dispersion and extremely high dielectric permittivity of colloids and biological tissue, Schwarz (1962) pointed out that small electric fields can move counter-ions surrounding the cell parallel to the cell surface but that any component of the field perpendicular to the surface will have negligible effect in comparison to the large radial field of the negatively charged cell. In typical experiments of the type considered here (Poo, 1981), this radial field is larger than the externally applied field at distances less than 10 nm from the cell surface. The movement of counter-ions along the surface can explain the large dielectric increment observed in colloidal suspensions, where the relaxation

time is approximately 2 msec for 1 μ particles (Schwarz, 1962; Schwan *et al.* 1962). For a 15 μ radius cell the relaxation time will still be much smaller than 1 second (Schwarz, 1962). These results show that the time required for the polarization of the counter-ion layer is small with respect to the duration of the externally applied field in membrane molecular electrophoresis experiments and an asymmetric counter-ion distribution will be established before the charged intramembraneous macromolecules begin to migrate. This asymmetric distribution produces an electric field at the cell surface that is opposite in direction to the externally applied field.

In the next section, a model is described which relates the electric field at the cell surface resulting from this polarization to the externally applied field and various physical parameters of the system.

2. Model

Consistent with experiments in the literature (Poo, 1981), the cell is assumed to be fixed on a surface. It is an immovable hemisphere with charged molecules embedded in or on the surface in the standard ways (Bretscher, 1976) and surrounded by an ionic milieu. The membraneous molecules are free to move parallel to the cell surface but any force

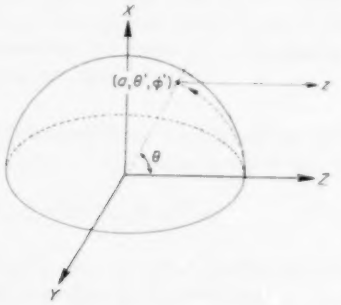

FIG. 1 Hemispherical model of a cell fixed on a flat surface. $\vec{E}_0$ is parallel to the z-axis. Two spherical coordinate systems are used, one centered at the center of the cell, using R, θ, ϕ, and one centered at the point of interaction on the cell surface (a, θ', ϕ') using r.

perpendicular to the surface will be counter balanced by various structural forces within the membrane. As in experiments, a homogeneous electric field ($\vec{E}_0$) is applied parallel to the surface on which the cells are fixed (see Fig. 1). The Z-direction is defined as the direction of this field. Outside the cell the additional potential due to the application of the external field,

V, as given by Schwarz (1962) is

$$V(R, \theta) = -E_0 R \cos\theta + f(K) a^3 E_0 \frac{\cos\theta}{R^2} \tag{1}$$

where R, θ are the radial and polar spherical coordinates, centered at the center of the cell of radius a and $f(K)$ is a function of the conductivities of the counter-ion layer and the cell surface. ($f(K)$ will be between 0 and $-0{\cdot}5$ for all reasonable cases with a value near the lower limit most likely.) $V(R, \theta)$ is a small perturbation to the intrinsic electrostatic potential.

The ions around the cell can respond much more rapidly to the field than the mobile, charged molecules within the membrane. The cell concentration is assumed small enough so the ionic double layers do not overlap and the current is carried between the electrodes by negative ions and positive ions outside the immediate counter-ion layer (at least a few nanometers).

The charge distribution outside the cell due to ions with a relaxation time that is short with respect to the duration of the externally applied field is:

$$\rho(R, \theta) = \sum_i \rho_{0i}(R)\, \mathrm{e}^{-q_i V(R, \theta)/kT} \tag{2}$$

after a suitable delay, for R near the cell surface where the sum i is over all types of mobile ions trapped near the cell surface, $\rho_{0_i}(R)$ is the charge distribution for a particular ion when no external field is applied, q_i is the charge of the ith ion, k is Boltzmann's constant and T is the absolute temperature.

The electrostatic interaction energy between this charge distribution and a point charge on the cell surface is,

$$W(a, \theta') = \int \rho(R, \theta) I(|\vec{x} - \vec{x}'|, D)\, \mathrm{d}\tau \tag{3}$$

where I is a unit interaction function that depends only on the distance ($|\vec{x} - \vec{x}'|$) between the two interacting units and the physical properties of the material (D), $\vec{x}' = \vec{x}'\ (a, \theta', \phi')$, on the cell surface, and $\vec{x} = \vec{x}\ (R, \theta, \phi)$, outside the cell, are position vectors. The integration is over all space outside the cell (τ). The component, parallel to the cell surface, of the electrostatic force on a charged macromolecule due to this charge distribution is

$$F_\theta = q' E_\theta = \frac{q'}{a} \frac{\partial W}{\partial \theta'} \tag{4}$$

where q' is the total charge of the interacting macromolecule including

both the charges on the molecule and the charges of ions that are so tightly bound they move with the molecules in small fields.

Equation (4) assumes that the variation in W is small over the dimensions of the macromolecule and that any contribution due to the orientation of the macromolecule about its center of mass is unimportant (Rabinowitz, Swissler & Rein, 1972). These assumptions then make it convenient to compare E_θ with E_0 to ascertain the relevance of counter-ion distribution polarization to the field induced motion of charged macromolecules on/in the cell surface.

In order to make this comparison it is necesary to specify $I(|\vec{x}-\vec{x}'|, D)$ and $\rho_{0i}(R)$. In recent quantum mechanical studies (Corongiu *et al.*, 1979) of the interaction of sodium ions with analogs of carrier molecules, a Lennard-Jones potential with an added coulomb term was found to adequately reproduce the interaction energies

$$I(|\vec{x}-\vec{x}'|, D) = \frac{-A}{r^6} + \frac{B}{r^{12}} + \frac{Cqq'}{r} \tag{5}$$

where $r = |\vec{x} = \vec{x}'|$ and A, B and C are fitted constants. In all but two of the 27 "classes" of atoms they considered, the coulomb term leads the interaction beyond 2 Å, and in all but one "class" the coulomb term leads the r^6 attractive term. For all "classes" of heavy atoms but one, the hard core repulsive term dominates at 1 Å and for the excepted class it dominates at 0·75 Å. This suggests that for distance greater than 2 Å a coulomb type interaction between ions and charged intramembraneous particles is appropriate and at smaller distances a hard core repulsive term is suitable. The latter can be most simply accomplished for the purposes of this study, with a non-zero lower limit of integration for the radial integral implicit in equation (3).

Corongiu *et al.* (1979) found the fitted constants C for 24 of the 27 "classes" are near unity. In that study the presence of water molecules was not considered. Near the cell membrane there are approximately 100 water molecules for each counter-ion. Further, only the interaction with a single ion was considered. The interaction with the solvent can be included by using a dielectric constant in the last term of equation (5), and inclusion of the effect from multi-ion interactions can be accomplished by using a screened potential (Morawetz, 1961).

For $r \geq 2$ Å

$$I = \frac{qq'}{\varepsilon r} e^{-\kappa r} \tag{6}$$

where ε is an appropriate dielectric constant, and κ is the screening

parameter, $\kappa = \kappa(\varepsilon)$. For r small with respect to cell size, ε is no greater than the dielectric constant of water and may be much smaller (Harris & Rice, 1954).

For $\rho_{0i}(R)$ we take a Boltzmann distribution for each type of ion

$$\rho_{0i}(R) = \rho_{\infty i}\, e^{q_i \Psi(R-a)/kT} \tag{7}$$

where $\rho_{\infty i}$ is the charge distribution for the ith type of ion far from any cells and $\Psi(R-a)$ is the unperturbed radial potential taken from Gouy–Chapman theory for these calculations. It is assumed that the excess positive charge at any point is the counter-ion distribution.

The problems with using the Gouy–Chapman theory have been discussed but it also has been observed that at least for lipid vesicles it provides a good description of some experimental results (McLaughlin, 1977). Its use is consistent with equation (2), but like the Lennard–Jones potential it does not take the interaction between ions into account. This emphasizes the importance of using the screened potential in equation (6).

It would be preferable to use a charge distribution that contained discrete charges under the influence of the various charged sites on the cell surface. However the surface charge distribution and local potential functions are unknown (even if the zeta-potential or total surface charge is available) and it is not clear at present how to obtain quantitative results from this approach. We have chosen equation (7) because the approximations in it are consistent with other similar discussions (McLaughlin & Poo, 1981), it is consistent with equation (2) and it provides a quantitative solution for the proposed problem.

3. Calculations and Results

At a time after the external field is applied that is long with respect to the relaxation time of the ions near the surface, but short with respect to any movement of the charged molecules within the surface, the electric field (E_θ), due to counter-ion distribution polarization, may be estimated at any point on the cell surface from equation (4) using equations (2), (3), (6) and (7). The charges more than a few nanometers away from that point make no contribution to the field at that point. Within the small relevant volume (i.e., a few nanometers from the point of interest) the charge distribution varies with the distance from the surface. After the field is applied the charge distribution varies parallel to the cell surface but since the radius of the relevant volume is much smaller than the radius of the cell, the variation in θ' within that volume will have a negligible effect on the final result. We also assume that $qaE_0 < kT$. Using this information to

expand the exponential in equation (2) and retaining only the largest term that contains E_0 we obtain,

$$E_\theta \text{ (due to ion polarization)} = \int_{\Delta}^{\Delta'} \int_0^{\Pi} \int_0^{\Pi} \rho_0(R) \frac{e^{-\kappa r}}{\varepsilon r} d\tau \frac{qE_0 \sin \theta'}{kT} + \ldots . \tag{8}$$

where ρ_0 depends on the distance from the cell surface and q is the charge of a counter-ion (now assumed to be a single type).

The integral may be performed in a spherical coordinate system (r, θ, ϕ) about the point of interest (a, θ', ϕ'), Δ is 2 or 3 Å and Δ' is sufficiently large so that equation (8) is independent of the choice of Δ'.

E_θ has been estimated by approximating the integral in equation (8) in two different ways. In each case a large number of hemispherical shells about the point (a, θ', ϕ') are summed and each shell is assumed to have a constant charge density. In case A the charge density is taken as the minimum value in that shell $(\rho(R) = \rho(R_{max}))$. In case B the charge density is taken as the value at the mean of R for that shell $(\rho(R) = \rho(\bar{R}))$.

Figure 2 gives the calculated values for E_θ in units of $E_0 \sin \theta$ as a function of surface potential in a 0·15 molar salt solution for $\Delta = 2$ Å, (the values for $A = 3$ Å differ by less than 10%). It can be seen that at all surface potentials of interest E_0 is a significant fraction of E_0 and at surface potentials of 50 mV or more E_θ may be greater than E_0 at angles near 90°.

For some values of E_0 used in the experiments considered, qaE_0 may be large enough that higher terms in the expansion in equation (8) will make a significant contribution to E_θ but the expansion itself is still valid. For example, the contribution of terms with higher powers of E_0 is less than 5% for a 15 μ cell in a solution of monovalent ions when the externally applied field is 1 V/cm. If the externally applied field is 10 V/cm, four higher terms each contribute significantly and their total contribution is approximately 75% of the leading term. When $qaE_0 \gtrsim kT$, E_θ will have explicit dependence on the radius of the cell and the angular dependence will be more complicated. On the side of the cell near the negative pole, the higher order terms have the same sign as the leading term while on the opposite side the sign of terms alternates. At the midline of the cell where the leading term is maximal the higher order terms make no contribution. This more complicated dependence on θ will not change the general conclusions of this paper.

The magnitude of E_θ decreases as temperature increases. As the temperature changes from 5°C to 37°C it changes by less than 15%. The ratio of E_θ for bivalent ions to E_θ for monovalent ions is approximately two. It

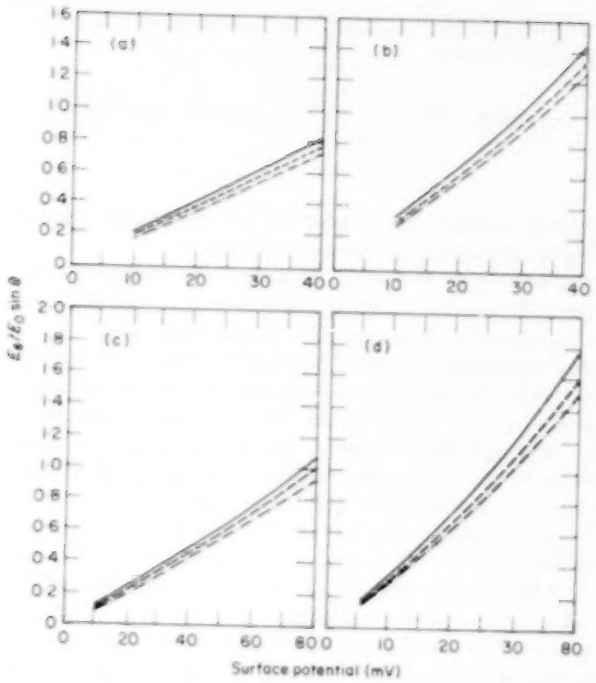

FIG. 2. The electric field due to counter-ion distribution polarization for monovalent and bivalent salts. Curves are shown for three temperatures and two ways of evaluating equation (8); —— = 5°C; - - - = 25°C; · - · - · - = 37°C. Case A is a minimum value. If $\rho(R)$ is chosen from the distribution of positive ions only, E_θ is larger by a factor of 3–1·2 for surface potentials of 10–80 mV. (a) Case A, $q = 2$. (b) Case B, $q = 2$. (c) Case A, $q = 1$. (d) Case B, $q = 1$.

increases from 1·8 to 2·1 as the surface potential increases from 10 to 40 mV.

4. Discussion

E_θ is a function of the externally applied field, position on the cell surface, the cell surface potential, the charge of the mobile ions and other physical parameters but it is independent of the radius of the cell for small applied fields (≈1 V/cm). It is opposite in direction to the applied field and for cells with a great enough surface potential, it could explain an accumulation of negatively charged molecules at the side of the cell nearest the negative pole. The change in sign of the total field with increasing surface potential

is qualitatively similar to the effect on the resultant force in the electro-osmotic model (McLaughlin & Poo, 1981), but the effects of ion distribution polarization will have a nonlinear dependence on surface potential at some potentials of biological interest (see Fig. 2).

E_θ is approximately of equal magnitude to E_0 and results from a slight shift of the centre of charge of the mobile counter-ions near the cell surface. Because we are considering times before the charged intramembraneous components begin to move, they retain their initial distribution, independent of θ and ϕ. The new total charge distribution has a dipole moment with a component parallel to the cell surface. If one makes the inherent assumptions in Gouy–Chapman theory and assumes the surface charge distribution is smeared out, the electric field near the cell surface due to the charges on that surface is radially directed and five orders of magnitude larger than the external fields (E_0) used in typical experiments on the migration of cell surface molecules. It can be seen that the perturbation by the external field causes only a very slight shift in the direction of the unperturbed field.

This study uses continous, macroscopic parameters to describe a system that is discrete if one considers it in detail. Some discussion of these macroscopic parameters is necessary. The numerical values in Fig. 2 depend on the dielectric constant of the media. The value of 78, the dielectric constant of free water, was used. If the water molecules near the cell surface are not free to orient in an electric field, a much smaller dielectric constant, perhaps as low as 5·5 would be appropriate (Hasted, Retson & Collie, 1948). It is far from clear what dielectric constant is appropriate for the problem being discussed. It may be best described as a function of position relative to the cell surface or particular surface charges. The dependence of E_θ on ε is both explicit and implicit as κ is also a function of ε.

The continuous charge density used for the counter-ion distribution may be seen as the average positions of discrete ions averaged over the time it would take a charged intramembraneous particle to move a significant distance (much longer than the relaxation time for the ions). Some ions may be specifically associated with sites on the membrane and may not be sufficiently described by a continuous charge distribution. These ions enter this analysis as part of the site, changing the surface charge of the cell and changing the charge of the intramembraneous particle. If these ions have some freedom with respect to the charged intramembraneous particles they will make a significant contribution to the force on that particle. Ionic charge distributions that are localized near charged sites on the membrane and are not initially spherically symmetric may be needed to explain experimental detail and will be the subject of further studies.

It is interesting to note here that the force due to counter-ion distribution polarization results from the movement of ions within the first few nanometers of the cell surface while the electro-osmotic forces are due to the movement of ions and water further from the cell surface. Both of these forces may play a significant part in determining the movement of charged membrane components. The actual numerical comparison of the force induced by counter-ion distribution polarization, to other forces on charged intramembraneous particles is complicated by factors previously discussed.

The striking aspect of the results described by Poo (1981) is that an electric field supplied by an external source causes some charged membraneous molecules to alter their lateral distribution in opposition to randomizing thermal forces. The change in potential energy of a monovalent ion, in solution near the cell surface, moving from one pole of a 15 μ cell to the other is smaller than kT, $2{\cdot}7\times10^{-2}$ eV, (a necessary conditon for the expansion in equation (8) to be convergent) in the experiments considered (Poo, 1981) but if the membraneous molecule is a polyelectrolyte that will not be the case. In a 5 V/cm field $q'E_0\,a$ is greater than kT for $q'=4$, a polyelectrolyte charge equal to four electrons. The present study indicates that the ionic environment of the cell plays a significant role in this phenomenon and suggests that the local ionic milieu of the migrating membraneous molecule influences the final distribution. Possible effects on molecular and cellular function due to this altered distribution may result from two sources. One, the migrating molecules are more (less) densely packed on one side of the cell than they would be with no external field. As a result, they will interact more (less) with each other, affecting cooperative functions between molecules and intercellular communication. Two, the local environment of the molecule may be changed by migration, affecting molecular structure and the availability of functional cofactors. Experiments of this type with specific functional endpoints will provide insight into the effects of electromagnetic fields on biomolecular function and ultimately on macroscopic biological systems.

5. Conclusion

An electrostatic force, that arises from the polarization of the counter-ion distribution near the cell surface by an externally applied field, will have a significant effect on the migration of charged membrane components. This force along with other forces that oppose the externally applied field, offers a possible explanation for the movement of these molecules in a direction not expected if only the external field is considered. The actual

movement of charged particles in the membrane is determined by the sum of various electrostatic, hydrodynamic and structural forces.

The difficulty in quantifying and comparing these forces results from an incomplete knowledge of the physical nature of the cell membrane and the difficulty in using macroscopic, continuous concepts to describe microscopic, discontinuous circumstances. Similar studies on more homogeneous and well specified systems, like lipid vesicles would be easier to quantify. Extrapolation to a more rigorous biological context would be necessary in order to consider the potential biological effects of electric fields.

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